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**Temperature-Induced Changes in Reversed-Phase Chromatographic Surfaces:
C7 and C8 Monomeric Ligands**

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TEMPERATURE-INDUCED CHANGES IN REVERSED-PHASE
CHROMATOGRAPHIC SURFACES: C7 AND C8 MONOMERIC LIGANDS

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Summary

The temperature-dependent retention of phenol and resorcinol on a monomeric C8 stationary phase, preconditioned with acetonitrile and then contacted with water, is investigated. These results are compared with the surface polarity of C7 and C8 monomeric ligands under similar conditions, the using the fluorescence vibronic band ratios of sorbed pyrene as a probe. Unlike polymeric stationary phases in the same size range, the solute elution and surface polarity of monomeric ligands indicate no persistent retention of the preconditioning solvent nor a distinct transition temperature for its accelerated loss. The results indicate that monomeric stationary phases are amorphous surface structures which do not achieve the same degree of conformational order as polymeric ligands; the results are consistent with previous studies of the shape selectivity of monomeric and polymeric phases. (JES)

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INTRODUCTION

The development of a comprehensive model for reversed-phase liquid chromatography has been challenged to understand the role of the alkylated surface in the selective retention of solute molecules. While changes in mobile phase dominate solute retention [1,2], the contribution of the hydrophobic surface to selectivity has been the subject of considerable investigation and interest [3-13]. A number of models have been proposed for the structure of monomeric stationary phase surfaces. A "brush" model, with alkyl ligands extended into the mobile phase, has been proposed [5] as a probable surface structure for monomeric alkyl ligands in reversed-phase liquid chromatography, which was a natural conclusion from intuitive thinking about the geometry of monomeric bonding. This view is being replaced, however, by models of a heterogeneous surface incompletely covered by alkyl ligands. Lochmüller and Wilder [4] produced evidence that, in the presence of aqueous mobile phase solutions, alkyl ligands avoid unfavorable interactions with the solvent and are aggregated into hydrophobic clusters. If the solute is sufficiently small to be contained within these clusters, the stationary phase appears as islands of non-polar material distributed on a polar silica surface. The organization of monomeric versus polymeric ligands, affected by differences in bonding to the surface, appears to influence shape selectivity of retention and depends on the degree of surface coverage and the geometry of the underlying substrate [11,12].

Distribution of solvent components into monomeric stationary phases has also been studied, and the resulting effects on solute retention have been described [6,9,10]. The presence of the organic modifier from the mobile phase is responsible for changes in the stationary phase volume [6,9] and for

changes in the environment of a solute sorbed to the bound layer [13,14]. The largest differences between the polarity of solute environments in monomeric versus polymeric stationary phases are observed when the organic modifier concentrations are small [14]. The intercalation of organic modifier into stationary phases may, in addition to changing the polarity of the interfacial environment, be responsible for changing the conformation of the alkyl ligands as well, as revealed by infrared spectroscopy [15].

While studying the effects of temperature and conditioning solvent on polymeric C8, C9, and C10 stationary with a totally aqueous mobile phase, Gilpin and co-workers [7] noted a hysteresis in the temperature dependence of solute retention, following preconditioning with organic solvent. They showed in a subsequent study that organic modifier, initially retained by the stationary phase when the mobile phase is abruptly changed from neat organic modifier to water, is released [8] when a sufficiently high temperature is reached. The temperature at which the solvent was released correlated with the alkyl chain length and functionality of the organic solvent [7,16]. In a recent investigation of the surface environment of C8 and C9 polymeric phases using the fluorescence from pyrene as a probe [17], an abrupt decrease in the polarity of the surface was observed to coincide with temperature at which the change in solute retention was observed. This observation that the mechanism for retention of organic modifier in the stationary phase was not physical entrapment since the alkyl ligands do not extend into the aqueous mobile phase upon its release. The unique temperature dependence of solvent retention in polymeric ligands, instead, requires that alkyl ligands have possess a degree of local order in the presence of the organic solvent which contributes to the stability of the solvated surface structure.



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To determine whether local ordering of bound ligands could play a role in temperature-dependent hysteresis effects in reversed-phase materials, the investigation of these effects is extended in the present work to C7 and C8 monomeric stationary phases. Following the methods of Gilpin and coworkers [7,8,16], solute retention is measured on a monomeric C8 column as a function of temperature, following preconditioning with acetonitrile and switching to a totally aqueous mobile phase. Measurements of the surface polarity of monomeric C7 and C8 surface polarity were made under identical conditions using the fluorescence of sorbed pyrene as a probe. Unlike polymeric stationary phase materials, neither the persistent retention of conditioning solvent nor a distinct transition temperature for its release are observed for monomeric phases. The results indicate a more disordered structure at the interface of these materials, which is consistent with the previous studies of their shape selectivity for retention compared to polymeric phases [11,12]

EXPERIMENTAL

Chemicals. Phenol and resorcinol were obtained from Aldrich and used as received. Pyrene was obtained from Aldrich and used after recrystallization from ethanol-water solution. Reversed-phase HPLC analysis of the compound using a 10 cm, C18 column revealed no resolvable impurities. Acetonitrile (HPLC grade) was obtained from MCB and Burdick and Jackson. Water was purified in house using a Corning still (MP-1) and a Barnstead Nanopure system. Monomeric C7, and C8 stationary phases for spectroscopic studies were prepared in house by reacting the dimethylchlorosilane with the appropriate terminal alkene in benzene in the presence of chloroplatinic acid as a catalyst. The resulting n-alkyldimethylchlorosilanes were bonded to 10 μm irregular silica

(Partisil-10) having a surface area of approximately $320 \text{ m}^2\text{g}^{-1}$ and a mean pore diameter of 96 Å before derivatization. The silica, dried for 24 hours at 165 °C, was refluxed with the silane reagent in dry toluene for 10 hours. After filtering and washing successively with dry toluene, ethanol, and water, the stationary phase was dried under vacuum. Carbon loading of the resulting phases was determined to be 8.3% and 8.9% for C7 and C8, respectively, by M-H-W Laboratories (Phoenix, AZ).

Chromatographic measurements. Retention measurements for pyrene under conditions of 100% aqueous mobile phase were carried out on a Beckman Model 110A HPLC using UV detection. The column used was 5 cm in length, obtained from DuPont, packed with C8 monomeric stationary phase on 5 µm silica gel. Flow rate was 1.0 ml/min, and deuterium oxide was used as the void volume marker. Before each experiment, the stationary phase was conditioned by passing large volumes of acetonitrile through the column (approximately 50 ml) at a flow rate of 1 ml/min. The mobile phase was changed to 100% water for the remainder of the experiment. Before any retention measurements were recorded, enough aqueous mobile phase was passed through the column to flush out the unretained organic solvent until a steady baseline was achieved. The column was in thermal contact with a circulating fluid bath (Haake, model L) and could be maintained at any temperature between 10 °C and 110 °C (+/- 0.25 °C) using a Haake temperature controller (model D3). The reported retention results were obtained from the average of four measurements with reproducibility being 3%, relative standard deviation.

In a typical experiment, the column would be raised from 20 °C to 65 °C and then cooled down to approximately 25 °C. Chromatograms were recorded every 10.0 °C. After recording a chromatogram at a particular temperature the

system was allowed to reach the next temperature and held there for either 10 or 20 minutes, to test the effects of thermal equilibration; during this period the solvent flow was stopped. Aqueous mobile phase at a flow rate of 1.0 ml\min was then pumped through the column for three minutes to remove any released organic modifier.

Spectroscopic sample preparation. The stationary phase was contained in a 1.5 mm ID precision bore quartz column (Wilmad Glass Co.). The quartz tube was held in place by a brass cuvette. Both the cell and the packing procedure have been described previously [18,14]. The stationary phase was preconditioned by passing large volumes of acetonitrile through the column (approximately 50 mls or 2000 column volumes) at a flow rate of 1 ml\min. Pyrene was introduced to the column in a 50:50 (V:V) acetonitrile:water mobile phase. The concentration of pyrene in the mobile phase was adjusted to a value of ca. 3.0 μ M, which gives a surface concentration low enough to avoid excimer formation (to assure that no probe-probe interaction are present). When the pyrene concentration on the column reached equilibrium (as determined by a steady-state fluorescence signal), the mobile phase was changed to 100% water for the remainder of the experiment. The contributions to the fluorescence signal from pyrene in the mobile phase were extremely small due to the high capacity factor conditions for 100% water mobile phase conditions. Based on the capacity factor for pyrene eluted by water on a C18 phase [19], correcting for the lighter carbon loadings of the C7 and C8 monomeric stationary phases studied here, results in a mass fraction of pyrene in the mobile phase less than 0.1%.

Fluorescence measurements. Emission spectra of pyrene sorbed onto the stationary phase were measured at 2.5 nm resolution on a Farrand model 201 spectrofluorimeter (Farrand Optical Co. New York, NY). The excitation

wavelength used was 335 nm. To obtain a measure of the stationary phase polarity, the intensity ratio of the third vibronic band to the highest energy, first band was calculated. The reported results were typically obtained from the average of 7 spectra. At a particular temperature, the precision of the vibronic band intensity ratios was normally 0.3% relative standard deviation. The brass cuvette, when placed in the spectrometer, was in thermal contact with the same circulating bath used to maintain the temperature of the chromatographic column. The temperature program used in the fluorescence experiments was the same as that used in the retention measurements, above.

RESULTS AND DISCUSSION

Retention behavior versus temperature. The temperature dependent retention of phenol and resorcinol on an acetonitrile-conditioned C8 monomeric stationary phase under totally aqueous mobile phase conditions (Figure 1) shows a distinctly different behavior compared to that was observed for polymeric surfaces [7,8,16]. In the C8 polymeric stationary phase case [7], the log of capacity factor for these solutes decreased linearly with $1/T$ as the column temperature is increased until an abrupt decrease in k' is observed at a characteristic temperature of 40.7 °C. After this discontinuous change in the value of k' , the slope of the $\ln k'$ versus $1/T$ is not changed. The results in Figure 1 for a monomeric C8 surface, however, show no discontinuous change in the value of k' , but an abrupt decrease in the slope of $\ln k'$ versus $1/T$ at a temperature of 49 °C. After this change in slope, the system follows lower curve in its $1/T$ dependence, as the column temperature is raised further and then lowered. Both test solutes exhibit the same response to temperature, the only difference being the initial values of k' . Unlike the polymeric

stationary phase, the temperature at which the column exhibits a change in retention characteristics is not constant, but depends on the amount of time between observations. A reduction in the time allowed for the column to adjust to a temperature change from 20 to 10 minutes decreases the slope of $\ln k'$ versus $1/T$, and shifts the point at which the curves changed slope from 49 °C to 60 °C; see Figure 2.

Despite the differences between these results and those reported for polymer phases [7], the changes in retention behavior can, nevertheless, be explained by loss of acetonitrile intercalated into the hydrocarbon layer. The bottom portion of the respective curves for phenol and resorcinol in Figures 1 and 2 are indistinguishable, and represent solute sorption to a surface that is free of acetonitrile. The $\ln k'$ versus $1/T$ relationship following the initial heating is reproducible until the column is reconditioned. The upper portion of the curve represents sorption to an interface which contains organic modifier; however, the sorbed solvent is continually being released into the mobile phase at a slow rate. When the time between retention measurements is decreased, the surface has released less acetonitrile at a given temperature, and the k' of phenol or resorcinol is higher. Unlike polymeric stationary phases which exhibit a time-independent transition temperature, the temperature at which the solvent is released from the monomeric surface depends on time which suggests a disordered structure for this surface phase. The behavior is very similar to the temperature dependence of a glass-transition of a disordered material such as an amorphous polymer or supercooled liquid [20], and differs from the melting of an ordered structure which occurs at a distinct, time-independent temperature.

Surface polarity. The temperature dependence of the microenvironment

polarity of C7 and C8 monomeric surfaces was also determined using the sorbed pyrene as a probe. The vibronic band structure of the fluorescence from pyrene is sensitive to the polarity of the molecule's local environment [21]. As the solvent polarity around pyrene is increased, the intensity of the vibronic origin of the weak, highest energy L_b transition (Band I at 374 nm) is increased due to symmetry-lowering perturbations from the solvent environment which allow mixing with the much stronger L_a transition dominating the third vibronic peak (Band III at 385 nm) [22]. As a result, the ratio of the intensities of the third and first peaks, III/I, varies inversely with increases in the polarity of microenvironment of the pyrene probe [21].

There is a strong similarity between the temperature dependent behavior of the surface polarity (see Figures 3 and 4) and that of the solute retention, described above. The gradual decrease in surface polarity, as the temperature of a freshly preconditioned column is raised, is consistent with the loss of organic modifier from the surface. Complete loss of modifier is characterized by a constant response of the pyrene probe that is independent of temperature. This latter behavior is in contrast to polymeric stationary phases [17] which exhibited a significant temperature dependence in their surface polarity even after the release of the preconditioning solvent. The residual temperature dependence of the polymer phase appears to be correlated with the change in density of the probe's surroundings with temperature which lowers the effective dielectric constant [17]. The lack of a response to temperature for the monomeric stationary phase following loss of conditioning solvent is consistent with a symmetry perturbation of pyrene's excited state which is interfacial in nature, and thus depends less on the density of its surroundings. This perturbation would thus correspond to adsorption of the probe and not

partitioning into a bulk phase, a sorption mechanism which agrees with the limited degree to which monomeric ligands can organize into a continuous phase mass.

As in the case of the retention measurements, the slope of the upper portion of the polarity variation with temperature is dependent on the time elapsed since solvent preconditioning. If the column is held at a constant temperature polarity measured as a function of time, the solvent release from the stationary phase can be monitored. If the column is heated to a temperature of 30 °C and held constant, the surface polarity gradually decreases over a period of one hour until the steady state condition is attained indicative of a surface depleted of organic modifier; see path (a) in Figure 4. Repeating the experiment at an elevated column temperature of 47 °C, the surface polarity was observed to reach a constant value in less than 20 minutes, following path (b) in Figure 4.

The temperature dependent rate of solvent loss suggests a kinetically controlled release of sorbed acetonitrile analogous to glass-transitions in amorphous materials [20]. The intercalated solvent in the surface layer is thermodynamically unstable and differs markedly from the polymeric surface phase, where solvent must be incorporated into an ordered structure which "melts" only after a specific transition temperature is reached [7,8,17]. These observations could relate to differences in the shape selectivity of polymeric and monomeric stationary phases. Wise and Sander [11,12,23] have showed that C18, polymer stationary phases grafted at high-carbon loadings on large-pore silica supports produce unusually large selectivity for planar solutes compared to monomeric phases. Drawing on an analogy to retention on liquid crystalline phases in gas chromatography, they conclude that such

polymeric phases are considerably more "ordered" than monomeric phases. The selectivity also requires a high density of alkyl ligands with a surface configuration produced only by polymeric bonding of the phase, consistent with a partitioning of solutes into an ordered structure. These selectivity results and the conclusions of Wise and Sander about the relative order of reversed-phase surface structures clearly agree with the differences between polymeric and monomeric phases in their temperature-dependent response to solvent preconditioning described above. Furthermore, their conclusions are consistent with Gilpin and Squires' observation that the temperature-dependent solvation hysteresis of the polymer phase requires a significant surface coverage by alkyl chains and that, above a critical coverage, the magnitude of the hysteresis increases with increasing % carbon [7].

A final, practical outcome of the present investigation could be a better understanding of preconditioning memory effects in monomeric reversed phase materials. Monomeric phases are noted for long equilibration times required to reach constant retention after a significant change in the mobile phase composition. It is likely that the evolution of retention behavior results from the kinetically-slow changes in sorbed organic modifier concentrations, due to the disordered surface. To overcome this problem and promote more rapid equilibration of the retention characteristics, one could raise the column temperature and speed up the rate at which the the surface approaches its thermodynamically-stable condition, as was shown in the results of the present study.

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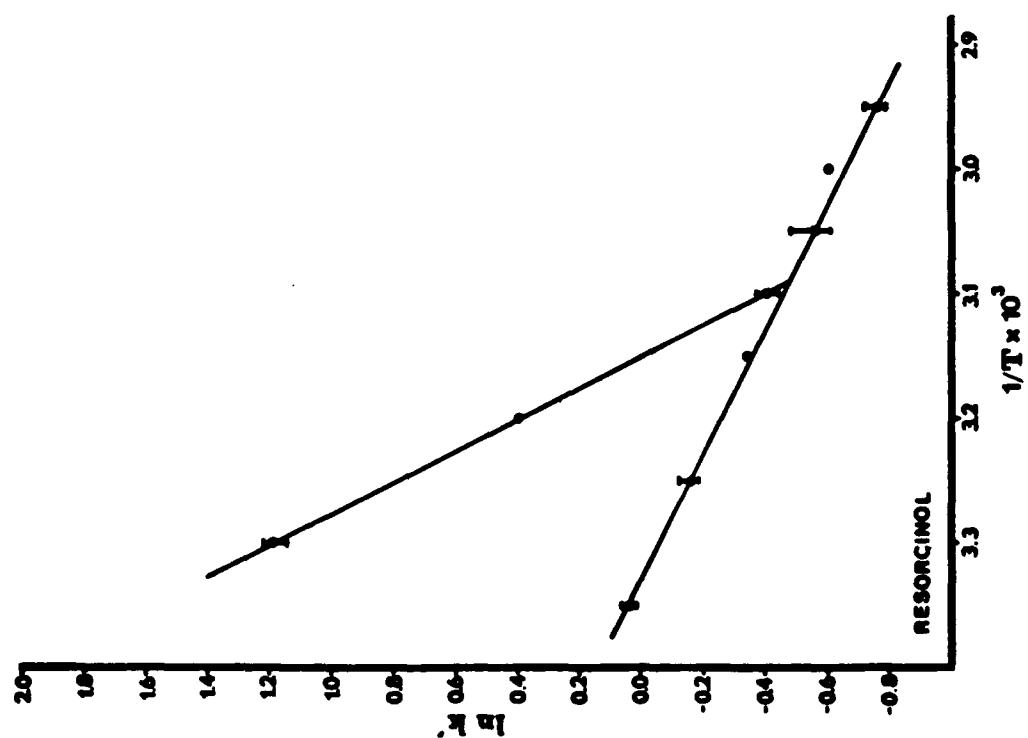
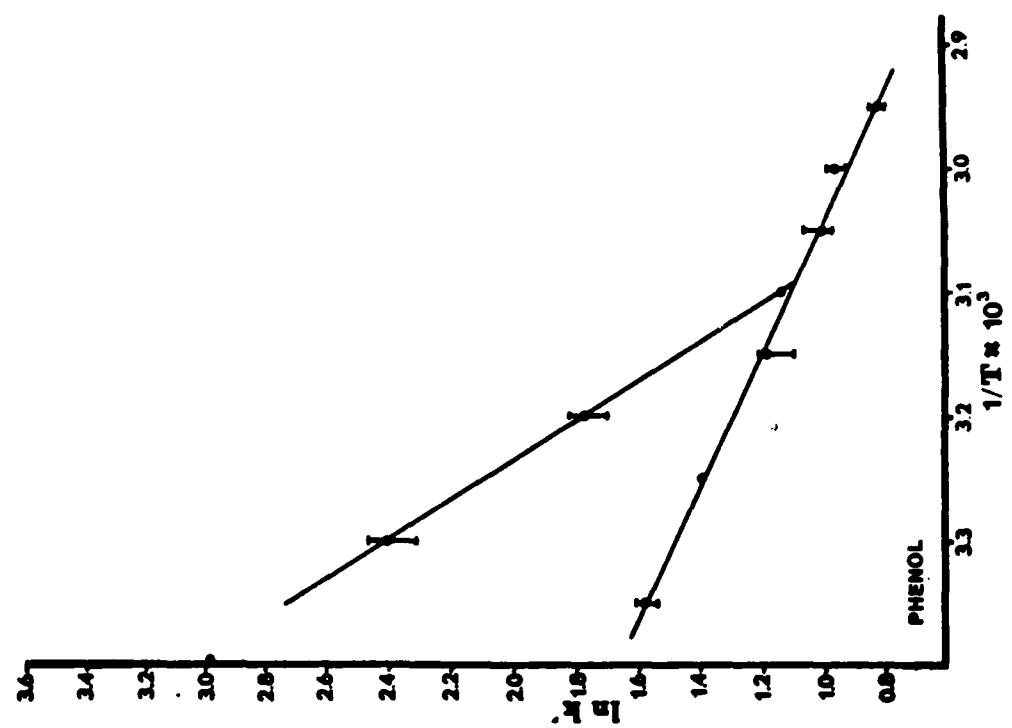
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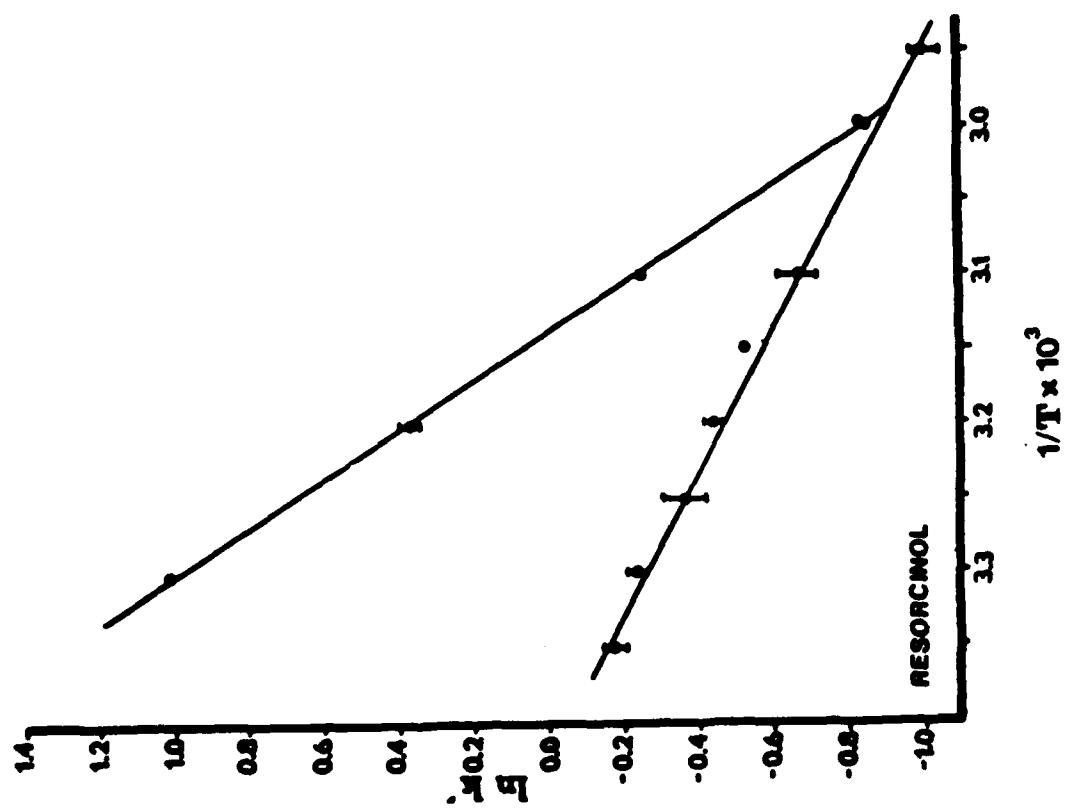
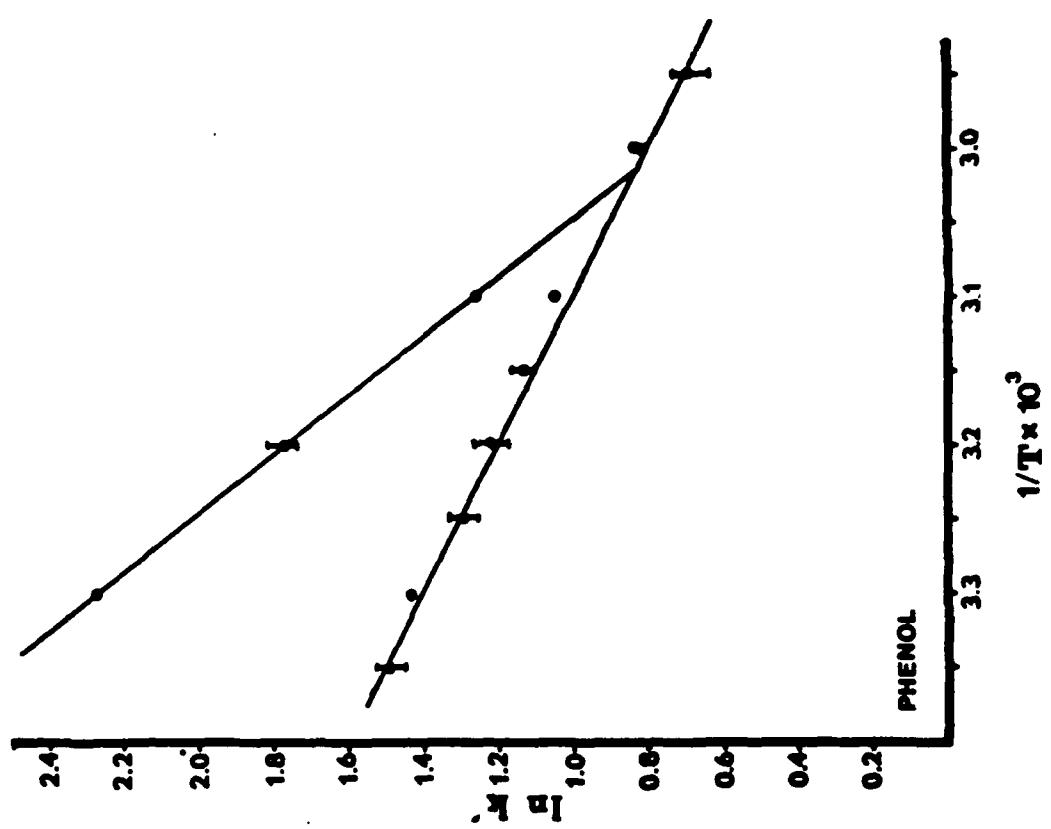
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FIGURE CAPTIONS

1. Temperature dependent retention of phenol and resorcinol on a monomeric C8 column eluted by an aqueous mobile phase. Twenty minutes equilibration time between the changing of temperature and measurement of retention. Upper curve represents the retention behavior immediately following preconditioning with acetonitrile. Lower curve represents the temperature dependence of retention following the excursion above 49 °C.
2. Temperature dependent retention of phenol and resorcinol on a monomeric C8 column eluted by an aqueous mobile phase. Twenty minutes equilibration time between the changing of temperature and measurement of retention. Upper curve represents the retention behavior immediately following preconditioning with acetonitrile. Lower curve represents the temperature dependence of retention following the excursion above 60 °C.
3. Temperature dependent microenvironmental polarity of pyrene sorbed to a C7 monomeric surface in contact with water. Upper curve represents the surface polarity immediately after preconditioning with acetonitrile. Lower curve represents surface polarity following the high temperature excursion as in Figures 1 and 2.
4. Temperature dependent microenvironmental polarity of pyrene sorbed to a C8 monomeric surface in contact with water. Upper curve represents the surface polarity immediately after preconditioning with acetonitrile. Lower curve represents surface polarity following the high temperature excursion
 - a) polarity change at a constant temperature (30 °C) follows this path; time required to change from the polarity of the solvated surface (upper point) to the unsolvated surface (lower point) is approximately 1 hour;
 - b) polarity change at 47 °C; time required to traverse this path is less than 20 minutes.





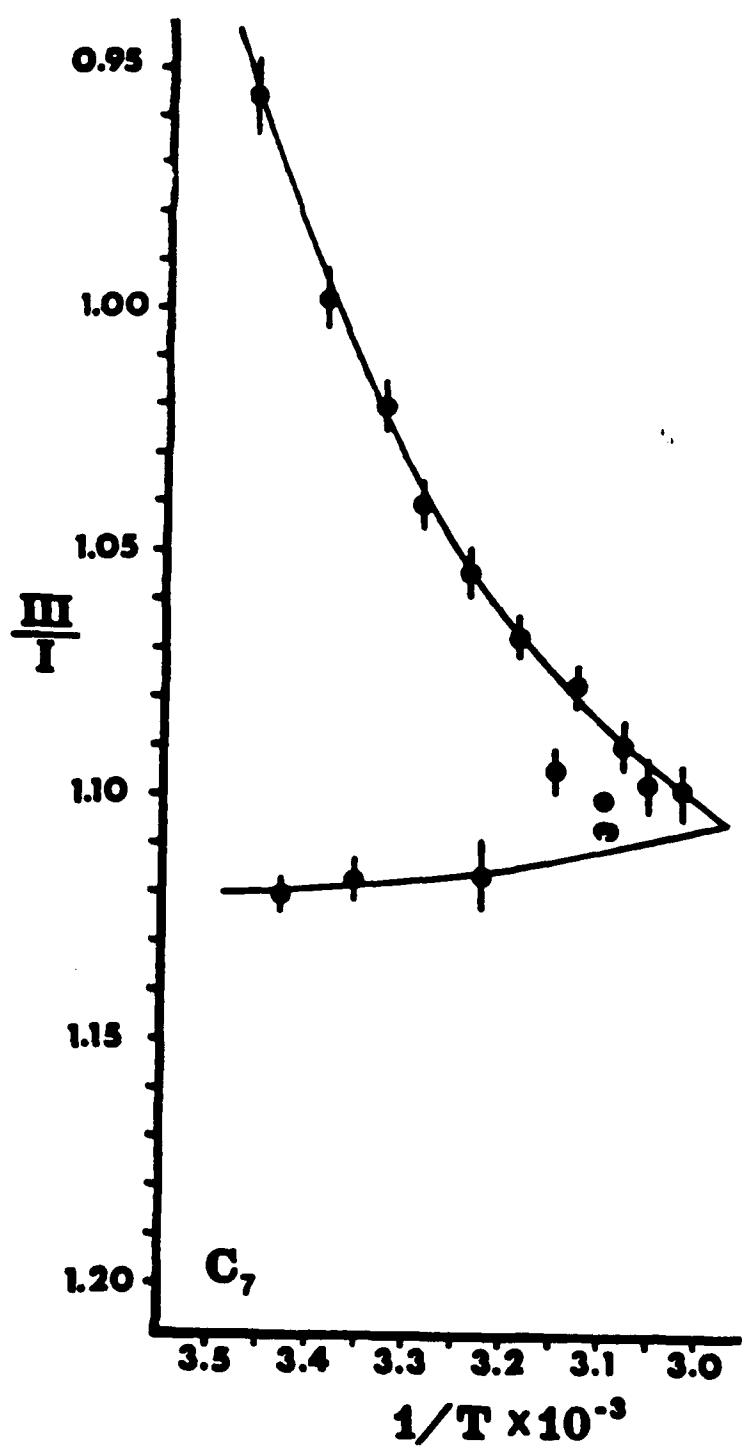


Figure 3

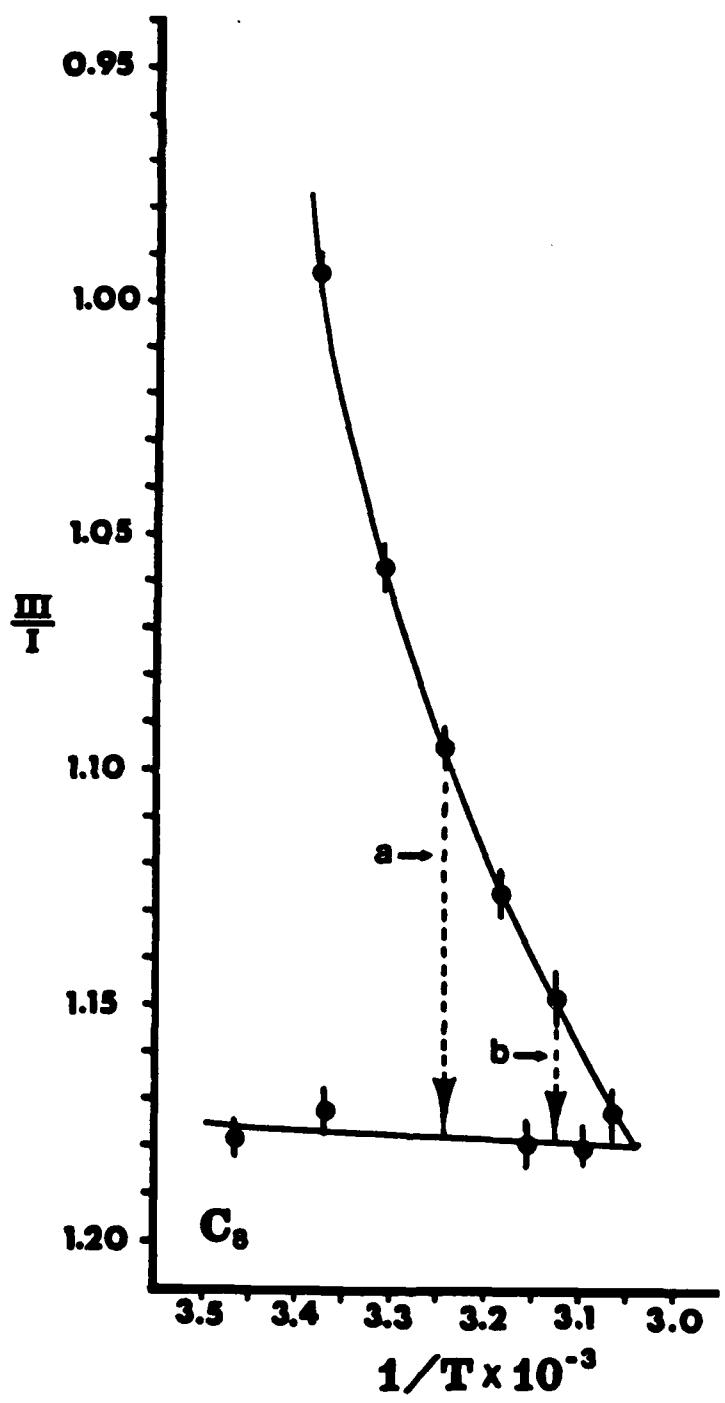


Figure 4